



# The Role of Quality Control and Reference Materials in the National Food and Nutrient Analysis Program (NFNAP)

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## Abstract

The USDA National Food and Nutrient Analysis Program (NFNAP) is achieving long-sought improvements in the National Nutrient Database through a comprehensive revision of scientific concept and technical approach, including representative nationwide sampling of foods, validated analytical methods, and an overarching quality control program. Food samples are shipped to a central laboratory, prepared, composited, and distributed to pre-qualified contract laboratories for nutrient analyses. Key features of the NFNAP quality control program are the approval process for analytical laboratories and inclusion of blinded, matrix-matched quality control materials in the sample stream to validate analytical results for the project.

Due to a well-known lack and high cost of commercially available certified reference materials (RM) for the full spectrum of food types and nutrients, twenty-six control composites (CC) comprising a range of food matrices have been developed for the NFNAP over the course of four years. Many of these composites have been analyzed at three or more laboratories for up to 40 nutrients, in conjunction with appropriate RM if available. In some cases results for RM and a laboratory's in-house control materials were acceptable, but those samples were of a different matrix and/or nutrient concentration compared to the test materials, and corresponding results for the externally supplied CC and/or RM were out of range. For example, in an assay of cholesterol in sausage and pepperoni pizza samples, data for the laboratory's in-house control sample (dried whole egg, ca. 2000 mg/100g cholesterol) was well within the laboratory's tolerance limits, while the value for a blinded matrix-matched CC (mixed food composite; ca. 10 mg/100g cholesterol) was well outside tolerance limits. In this way we were able to recognize errant data that may otherwise have gone undetected. These findings highlight the importance of submitting independent, blinded, food-specific control samples along with test samples to commercial laboratories for validation of analytical results.

## Objective

- To present results illustrating the key role of quality control samples in the National Food and Nutrient Analysis Program (NFNAP).

## Overview of the National Food and Nutrient Analysis Program (NFNAP)

- Goal:** Update and increase the quality of data in the USDA National Nutrient Database for Standard Reference.
- Sampling Plan:** Approximately 1,000 "Key Foods" have been identified based on contribution to overall nutrient intake in the U.S. population. These foods are targeted for analysis in NFNAP<sup>1,2</sup>.
- Scope of Sampling:** Foods have been sampled from nationwide retail outlets and composited at Virginia Tech. To date these include at least 15 types of beverages, 28 baked goods, and snack foods, 14 dairy products, 97 fast food items, 85 fruits, vegetables and juices, 28 grain products and cereals, 17 meat, fish and eggs, 15 nuts and nut butters and 49 miscellaneous foods (e.g. canned stews, soups, candy bars, spices), including multiple brands of products and different preparation of certain items (e.g. cooked and uncooked vegetables, pasta and eggs).
- Sample Shipment and Nutrient Analysis:** After compositing, food samples are grouped into batches with matrix-matched control composites and/or standard reference materials. Samples are shipped to contract laboratories for nutrient analysis. Nutrients assayed include: proximates, sugars, fiber, minerals, vitamin C, thiamin, riboflavin, niacin, pantothenic acid, vitamin B6, folate, vitamin B12, retinol, vitamin E, vitamin D, vitamin K, fatty acids, sterols, amino acids, caffeine, carotenoids, anthocyanadins, flavonoids, hydroxyproline, choline and fluoride.
- Figure 1 presents an overview of sample and data flow in the NFNAP and the role of control samples in the external NFNAP quality control program implemented to validate nutrient concentrations assayed in a wide variety of food samples at multiple laboratories over the course of five years.

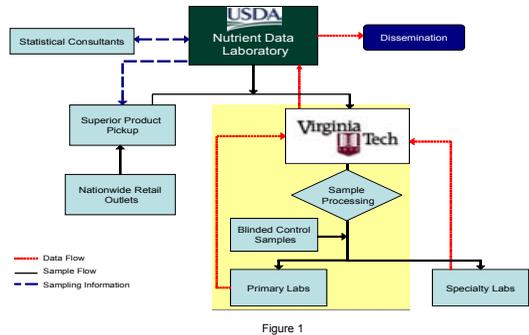


Figure 1

## Role of Quality Control and Reference Materials in the NFNAP

- Monitoring assays of NFNAP samples over period of several years
- Blinded control samples included with samples sent to laboratories:
  - Commercial reference materials (RM) (accuracy check) are sent only for critical nutrients.
  - Matrix-matched control composites (CC) developed as part of NFNAP are sent for all nutrients analyzed in the food samples.
    - monitor inter-laboratory variability
    - monitor within-laboratory precision

## Preparation of Control Composites

- Select foods of similar matrix relative to those planned for NFNAP sampling.
- Prepare homogenous composites of locally procured foods.
- Dispense ~200-1000 aliquots. Store at -60±5°C, under nitrogen, protected from light, in sealed glass jars with Teflon-lined lids.
- Table 1 summarizes NFNAP control composites that were prepared at Virginia Tech. These composites were designed to be similar in food composition and nutrient content relative to categories of foods sampled and analyzed for the NFNAP. Aliquots of these samples were included, blinded, in batches of the NFNAP foods sent to external laboratories for analysis throughout the NFNAP study for the wide variety of foods that have been analyzed for the NFNAP.

NFNAP Control Composites

Composite Name (number)	# Samples Generated	Foods Included	Foods Analyzed With
Baby Food (C0001P)	110	Commercial Beef Baby Food	Shrimp Stew, Beef/Chicken Hot Dogs, Frozen Chicken Nuggets/Strips, Frozen Chicken Tenders, Canned Chunk Light Tuna in Water, Egg Whites/Yolk Yolks, Orange Soup/Tuna Filets, Canned Cans, Canned Shrimp, Whole Eggs, Fast Food Chicken Nuggets, Whole Eggs (Raw/Hard Cooked/Fried)
Vegetable (C0001)	792	Baby Food Green Beans, Baby Food Carrots, Canned Green Peas, Frozen Winter Squash, Green Juice, Canned Spinach, Canned Asparagus, White Potatoes, Green Chili Peppers	Fresh, Frozen, and Canned Fruits and Vegetables
Mixed Food (C0802U)	408	Canned Chili (w/ and w/o Beans), Canned Spaghetti (w/ and w/o Meat), Canned Beef Stew, Frozen Pot Pie (Chicken and Turkey), Frozen Beef & Bean Burritos, Frozen Low-Fat Cheese Lasagna, Frozen Lasagna w/ Meat, Frozen Sausage Pizza, Frozen Pepperoni Pizza, Frozen Vegetable Pizza, Frozen Cheese Pizza	Canned Chili/Beef Stew, Frozen Cheese Pizza, Frozen Lasagna w/ Meat, Canned Spaghetti, Frozen Pepperoni Pizza, Frozen Sausage & Pepperoni Pizza, Frozen Chicken/Turkey Pot Pie, Frozen Meat & Vegetable Pizza, Frozen Cheese Lasagna, Frozen Beef & Bean/Ban & Cheese Burritos
Mixed Food II (CV10K9)	865	Canned Chili (w/ and w/o Beans), Canned Spaghetti (w/ and w/o Meat), Canned Beef Stew, Frozen Pot Pie (Chicken and Turkey), Frozen Cheese Pizza, Frozen Lasagna w/ Meat, Frozen Battered Fish Fillets, Frozen Pepperoni Pizza (distilled deionized water to facilitate blending)	Fast Food Sandwiches, Fast Food Tacos, Frozen Breaded Fish Sticks, Fast Food Pizza
Bread (C0802)	336	White Bread, Whole Wheat Bread, French Bread, Italian Bread, Seven Grain Bread	Dry Rice, Dry Pasta, White Bread, Hamburger/Hot Dog Rolls, Corn/Flour Tortillas, Salines, Plain/Seasoned Bread Crumbs, Toasty Peanut-Butter Filled Crackers, Cheese on Cheese/Peanut Butter on Cheese Crackers, Air-Popped Popcorn/Kernal Popcorn, Whole Wheat Bread, Instant Flour, Pretzels, English Muffins, Bagels, Oatmeal, Popcorn, Microwave (Regular & Reduced Fat), Conmodity Bakery Mix
Bread II (CV12B0)	480	Refrigerated Biscuits, Corn Muffins, Whole Grain Bread	Wheat Bread, French Bread, Uncooked Egg Noodles, Corn Taco Shells, Dinner Rolls, Refrigerated Biscuits, Degreased Yellow Corn Meal
Starchy Vegetables (CV12B2)	480	All-Free Canned Refried Beans, Cooked Potatoes, Baby Food Sweet Potatoes, Baby Food Corn	Navy Beans (Raw and Cooked), Canned Baked Beans, Canned Kidney Beans, Fast Food Hash Browns, Frozen French Fried Potatoes/After Tots
Snack Food (C0001Q)	144	Potato Chips, Pretzels, Cheetos, Corn Chips, Crackers	Shi-Popped Popcorn, Tortilla Chips/Potato Chips, Corn Chips/Cheetos, Tater Tots, French Fries
Salad Dressing II (C0001G)	144	Regular Ranch Dressing	Regular/Light Ranch Dressings, Canned Beef Gravy, Canned Chicken Broth, Sour Cream, Fast Food Milkshakes
Cheese II (C0001V)	240	Swiss Cheese, Cheddar Cheese, Low Moisture Part-Skim Mozzarella Cheese, Provolone Cheese, Monterey Jack Cheese, Cream of Chicken Soup, Tomato Soup	Sheddar Cheese, Whole Milk Mozzarella Cheese, Swiss Cheese Slices/Low Moisture Part-Skim Mozzarella, Whole Milk, 2% Thru/5am Milk
Margarine (C0802Q)	245	80% Fat Margarine (Soybean Oil), 70% Fat Margarine (Corn Oil), 70% Fat Margarine (Soybean Oil)	80% and 70% fat margarines
Oil (C0807H)	100	Peanut Oil	Corn/Olive Oil, Vegetable Oil
Pasta/Rice (C0809H)	144	Soaked Instant White Rice, Cooked, Cooked Extra Long Grain White Rice, Cooked Orzo	Soaked Rice, Cooked Pasta, Cooked Egg Noodles, Pinto Beans, Dried (Raw and Cooked) Cooked Orzo
Tomato (C0809W)	144	Tomato Puree, Tomato Sauce	Dried Apricots, Canned Tomato Paste/Puree/Sauce, Pasta Sauce (No Meat), Tomato Ketchup, Beefed Saus, Canned Whole Tomatoes in Juice
Orange Juice (C0814Z)	96	Pulp Free Orange Juice	Refrigerated Orange Juice, 100% Apple-Orange/Orange Juice (Juice Box), Orangeapple Juice, Cranberry Juice Cocktail, Pinaisoppe Juice, 10% Juice Drinks, Frozen Orange Juice Concentrate
Cake (CV10K0B)	432	Chocolate Cake, Frozen Pancakes	Frozen Pancakes/Waffles, Cake Mixes, Breakfast Cereals, Doughnuts, Blueberry Muffins, Chocolate Cupcakes, Taster Pastries, Fast Food Breaded Pastries, Graciosa Bars
Peanut Butter II (CV10K3E)	192	Creamy Peanut Butter	Nuts, Chocolate/Vanilla Frosting
Water (CV10K0A)	96	Bottled Water	Bottled Water, Fast Food Ice
Soda (CV10K0B)	173	Colas, Lemon-Lime Soda	Carbonated Beverages (Sodas), Ready-to-Drink Teas, Frozen Popsicles, Canned Peaches (Drained Liquids), Light Corn Syrup, Sports Drinks, Jello, Beer (Regular & Lite), Wine, Red & White, Instant Powdered Tea
Chocolate Milk (C0120V)	432	Chocolate Milk	Chocolate Milk, Fast Food Milkshakes, Pudding, Yogurt, Frozen Yogurt, Vanilla/Chocolate Ice Cream
Chocolate Candy (CV12K6)	288	Milk Chocolate Candy Bars	Milk Chocolate Candy, Baking Chocolate

\* Composites highlighted in light yellow are control materials selected for discussion.

Table 1

## Validation of Control Composite Homogeneity

- Homogeneity of each control composite is validated by analysis of indicator nutrients (e.g. moisture, total fat, ash) in aliquots drawn from across the dispensing sequence.
- Figure 2 illustrates data used to verify the homogeneity of two control composites.

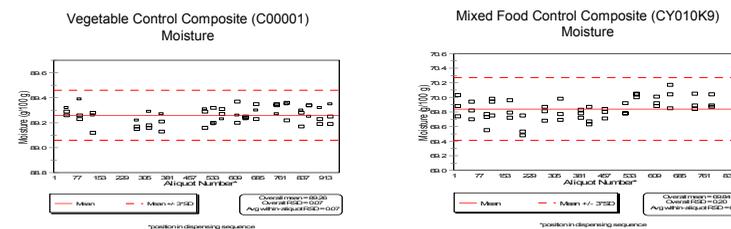


Figure 2

## Validation of Accuracy of Control Results via Certified Reference Material Cross-Validation of Results Among Labs Using Control Composite

The accuracy of data for nutrients in control composites could be verified in cases in which certified reference materials of similar matrix were available. In these instances, running the control and reference materials in parallel at one or more labs allowed establishment of tolerance limits for nutrient concentrations in the control composite, so that the control composite could be used to monitor and relate data obtained at multiple laboratories. Figures 3 and 4 are examples of results obtained for potassium and cholesterol in selected control composites (see Table 1 for description of composites).

Potassium in Vegetable Control Composite (C00001) Control to Reference Material Link

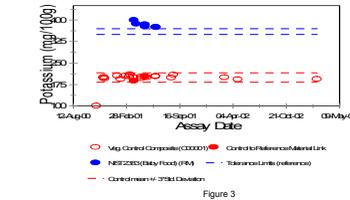


Figure 3

Cholesterol in Beef Baby Food Control Composite (C0001P) Control to Reference Material Link

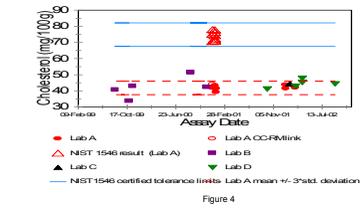
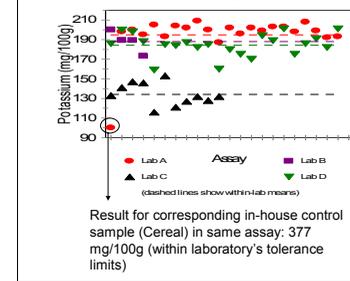


Figure 4

## Matrix-Specific Control Samples Indicate Assay Deviations Not Detected by Laboratory In-House Control Samples

- Matrix-specific NFNAP control composites sent blinded with test samples were able to indicate matrix specific assay deviations that were not detected by the laboratory in-house control materials in some cases in which the lab's control was a very different matrix or had a widely different nutrient concentration relative to the samples that are being analyzed.
- Below are three examples of how blinded NFNAP control composite samples were used to accept or reject data received from external laboratories.

Potassium in Vegetable Control Composite (C00001) Sent with NFNAP Fruit and Vegetable Samples

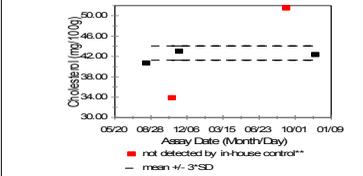


Result for corresponding in-house control sample (Cereal) in same assay: 377 mg/100g (within laboratory's tolerance limits)

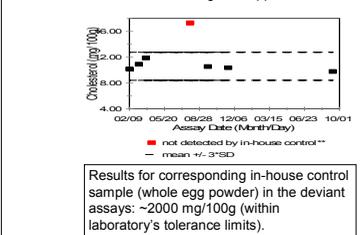
### Action:

- Rejected data for NFNAP lettuce and pear samples analyzed in same batch with deviate control value; new aliquots were sent for re-analysis.
- Rejected all data for potassium in fruits and vegetables assayed at Lab C.
- Link between data for NFNAP vegetable control composite and reference material (NIST 2383) of similar matrix allowed use of a control composite to check accuracy as well as precision.

Cholesterol in Beef Baby Food Control Composite (C0001P) sent with NFNAP Meat Frankfurter Samples



Cholesterol in Mixed Food Control Composite (C9802U) sent with NFNAP Sausage & Pepperoni Pizza



### Action:

- Rejected data for NFNAP pizza and frankfurter samples assayed in batches with deviation in NFNAP control composite (■); new aliquots were sent for analysis.

## References

- Haytowitz, D. B., Pehrsson, P. R., Holden, J. M. (2002). The Identification of Key Foods for Food Composition Research. *Journal of Food Composition and Analysis*, 15(2), 183-194.
- Haytowitz, D. B., Pehrsson, P. R., Holden, J. M. (2000). Adapting Methods for Determining Priorities for the Analysis of Foods in the Diverse Populations. *Journal of Food Composition and Analysis*, 13(4), 425-433.

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